said portion of the mixture of adaptor terminated fragments serves as a template to create a mixture of 3'-flanking VNTR amplimers

25. [Once amended] A method for diagnosing a trait of interest comprising the step of identifying an allele which is linked to a trait of interest according to the method of claim 22, wherein said molecules of nucleic acid are contacted with a [Use of the] portion of genomic DNA as claimed in claim 14 [in a diagnostic assay].

In the abstract:

Please add the enclosed abstract page 97 attached hereto as Appendix B.

In the Sequence Listing:

Please add the enclosed paper copy of the Sequence Listing.

REMARKS

I. Status of Claims

Claims 1-27 are pending in the instant case and stand variously rejected under rejected under 35 U.S.C. §101, 35 U.S.C. §102, 35 U.S.C. §103 and 35 U.S.C. §112, second paragraph. Exhibit A shows the claims as they would appear after entry of this amendment. Applicant believes the response and amendments presented herein overcome these rejections and place the instant case in condition for allowance. An indication of such favorable action is solicited from the Examiner.

II. Formalities

A. Sequence Listing Requirements

The present application contains sequence disclosures as defined in 37 C.F.R. 1.821(a)(1) and (a)(2) and as such is required to comply with the requirements of 37 C.F.R. 1.821 through 1.825. In order to bring the instant specification into compliance with the aforementioned rules, included herein is an amendment directing entry of sequence identifiers into the specification, and attached hereto are paper and computer readable copies of the Sequencing Listing, and a statement that the content of the paper and computer readable copies are the same and include no new matter. Applicant requests that these documents be made of record in the instant application.

B. Oath/Declaration

Applicant submits herewith a declaration claiming priority to EPO 98/42867 filed 21 March 1997 and EPO 82/42867 filed 21 March 1998. This declaration addresses the Examiner's comments regarding the oath/declaration in the instant case.

C. Certified copy of Priority documents

Applicant is in the process of obtaining a certified copy of EPO 98/42867 and will forward the same to the Patent and Trademark Office as soon as possible.

D. Specification Objections

The amendment presented above provides that the abstract commences on a separate sheet in accordance with 37 C.F.R. §1.52(b)(1).

E. Claim Objections

The Examiner objected to Claims 19 and 20 under 37 C.F.R. §1.75(c) as being improper dependent claims for failing to further limit the subject matter of a previous claim. Applicant has amended claim 19 to refer to claim 17. Applicant believes this amendment places the claims in proper dependent form.

III. Rejections under 35 U.S.C. §101

The Examiner rejected claims 11 and 12 under 35 U.S.C. §101 as allegedly claiming non-statutory subject matter. More particularly the Examiner found that the claims as written read on naturally occurring genomes. Applicant thanks the Examiner for the suggestion that amendment to include language at the beginning of the claims such as "An isolated portion of genomic..." would obviate the instant rejection. Applicant has amended the claim in accordance with the Examiner's suggestion and request that the rejection be withdrawn.

Claims 1-10, 21 and 25-27 are rejected under 35 U.S.C. §101 because the claimed recitation of a use without setting forth steps of the process result in an improper recitation of a process. The amendment presented above positively recites steps involved in the process and obviates this rejection.

In light of the above comments and amendments, applicant requests that the rejection based on 35 U.S.C. §101 be withdrawn.

IV. Rejections under 35 U.S.C. §112, second paragraph

The rejection of claims 1-10, 21 and 25-27 under 35 U.S.C. §112 (second paragraph) should be withdrawn in light of the amendment of the claims to set forth steps in place of a "use."

The rejection of claims 9, 10, 22-24, 26 and 27 should be withdrawn as it is clear to those of skill in the art that the language "of those" refers to the "at least one VNTR allele and its flanking sequence," that is both the VNTR allele and its flanking sequence.

The rejection of claim 13 may be withdrawn in light of the foregoing amendment of that claim.

V. Art-Based Rejections under 35 U.S.C. §§102 and 103.

A. The Rejection of Claims 11-15 and 25 Under 35 U.S.C. §102(b) Over Morgante Should be Withdrawn.

The rejection of claims 11-15 and 25 under 35 U.S.C. §102(b) over Morgante *et al.*, WO 96/17082 should be withdrawn because the cited reference does not disclose every element of the claims, including claim 11 as amended.

Figure 11 of WO 96/17082 does not show a portion of genomic DNA as characterized in the claims. Figure 11 shows a single VNTR, the sequence of which allows locus specific primers to be designed for subsequent analysis, and which does not constitute a mixture of VNTR alleles representative of one or more members of a species of interest.

Specifically, Morgante et al., disclose a process in which the genomic DNAs of two individuals are separately restricted and ligated to adapters from which polymorphic molecules containing microsatellite sequences are amplified. This process is described as "selective amplification of microsatellite polymorphic loci" (SAMPL) and is depicted in Figure 1a. Subsequently, the amplified products of the two subjects are resolved in parallel by polyacrylamide gel electrophoresis, discrepancies in the migration patterns of the labeled products indicated polymorphic variation.

In a preferred embodiment of Morgante *et al.*, two or more restriction enzymes are employed to restrict genomic DNA (page 52, line 6). This permits the ligation of different adapters to the ends of each restriction fragment. Another preferred embodiment describes the same principle (page 52, line 9). Using primers complementary to the adapter sequences together with a primer complementary to a microsatellite sequence, polymorphic molecules are amplified. If the label is carried by an adapter primer, a significant amount of background signal is created (page 56, line 34). This is because many genomic DNA fragments are capable of at least linear amplification regardless of the presence or absence of target microsatellite sequences (page 57, line 8). This problem would be compounded if only a single adapter had been ligated to the restriction fragments since all fragments would be capable of exponential amplification irrespective of the presence of target sequence. As such, it is the microsatellite primer that the Morgante et al., authors prefer to label (page 57, line 16) in order to generate a more discrete electrophoretic pattern of amplified products.

If polymorphic variation between two individuals is detected by discrepancy in electrophoretic separation of their amplified products, the appropriate band may be cut from the gel, cloned and sequenced in order to learn the identity of the microsatellite locus responsible (Morgante et al., page 68, line 5). The sequence generated would contain a portion of microsatellite, one of its flanking sequences and the adapter sequence to which the parent genomic fragment had been ligated, as shown in figure 1a. With the sequence of nucleotides flanking the microsatellite deduced, a primer can be designed to amplify the anonymous adjacent flanking the microsatellite deduced, a primer can be designed to amplify the anonymous adjacent flanking region from the parent genomic DNA fragment (Morgante et al., page 68, line 6). This is achieved using the "sequence design locus specific flanking primer" together with the appropriate primer that is complementary to the adapter on the opposing fragment end (page 68,

line 10). The amplified product is cloned and sequenced, thereby providing means to design a second "sequence design locus specific flanking primer (page 68, line 12). With these locus-specific primers, only the target locus will amplify from genomic DNA without concomitant generation of background signal, and they can be used to genotype samples from different individuals at this locus by conventional means. This process is depicted in Morgante et al., figure 11.

In contrast to the method of Morgante et al., genomic DNA is fragmented with one or more restriction enzyme in the method of Applicant's Invention. Subsequently, not only are the fragment ends provided with a single adapter that ligates to both ends of all fragments, but they are also provided with termini that prevent 3' strand extension by a DNA polymerase. This is of particular importance because all molecules in this form are inert during PCR regardless of the presence of adapter primer. Unlike the method of Morgante et al., therefore, neither linear nor exponential amplification of fragments is achieved and there is an absence of background generation of products. Only when a primer is introduced that has been designed against a target microsatellite sequence can polymerization occur. The microsatellite primer anneals to any fragments that contain a complementary sequence. The annealed microsatellite primer is extended to the fragment end, thereby creating a binding site for the adapter primer. The adapter primer anneals to the newly synthesised strand in the following PCR cycle and is extended. Exponential amplification occurs during further PCR cycles between the adapter primer and the microsatellite. This results in the en masse amplification of one flanking region of all loci in the genome containing sequence complementary to the microsatellite primer. At the end of PCR, the amplified products are digested to remove the microsatellite sequences from their ends, after which they are hybridised to genomic fragments bearing adapters and 3' termini derived from pools of individuals that possess or lack a hereditary trait of interest. The fragments

of these pools are inert to PCR by virtue of their 3' termini. However, the previously amplified microsatellite flanking regions that have annealed can be extended to generate binding sites for the adapter primer. During the following cycle of PCR, the adapter primer anneals at its newly created binding site and is extended along with entire fragment length to generate a binding site for the adapter primer at the opposing end. During further cycles of PCR, exponential amplification of microsatellite loci occurs en masse. It should be noted that microsatellites at numerous loci are amplified and the alleles that are generated reflect the genotypes of those individuals contributing genomic DNA to each of the phenotype specific pools. Genomic fragments that failed to hybridise are inert and no background is created. Consequently, the product consists "essentially of a representative mixture of alleles of a chosen microsatellite sequence and their flanking regions on both sides". These representative mixtures of alleles are subjected separately ro reiterated mismatch cleavage to enrich the most frequent allele at each of the numerous loci. Finally, having provided one of them with nuclease resistant fragment ends, or other described methods, the allele pools are hybridised and processed further to identify microsatellite loci at which allele frequency disequilibrium exists.

Applicant submits that the Section 102 rejection of claims 11-15 and 25 over Morgante et al., should be withdrawn because of the distinct differences between the Invention and WO 96/17082, and because of the radical departure of the Invention from conventional locus-by locus genotyping using conventional means. Moreover, the specific and deliberate wording of claim 11. In particular, "consisting essentially of" infers that the fragments of genome that do not contain the target microsatellite sequence have been eliminated through their failure to amplify at all, thereby avoiding background amplification of DNA. In addition, the alleles are "representative" of the genotypes of individuals contributing DNA to the phenotype-specific pools Also, "a chosen VNTR sequence" refers to the nature of the chosen repeated

nucleotide motif that may be present at many thousands of loci in the genome, all of which may be amplified en masse according to the invention. For these reasons, the rejection of claims 11-15 and 25 should be withdrawn.

B. The Rejection of Claims 16 and 22-23 Under 35 U.S.C. §102(b) Over Nelson et al. Should be Withdrawn.

The rejection of claims 16 and 22-23 under 35 U.S.C. §102(b) should be withdrawn because Nelson does not disclose all elements of the claims. Specifically, Nelson only treats genomic DNA, while the method of claim 16 involves nucleic acids which consist essentially of a mixture of polymorphic alleles. Moreover, with respect to claim 22, the method of Nelson is only used where the DNA is from individuals manifesting the same trait.

C. The Rejection of Claims 17, 18, 24, 26 and 27 Under 35 U.S.C. §103(a) Over Nelson et al. in combination with Grist et al. and Aldhous Should be Withdrawn.

The rejection of claims 17, 18, 24, 26 and 27 Under 35 U.S.C. §103(a) over Nelson et al., in combination with Grist et al. and Aldhous should be withdrawn because Nelson fails to teach the subject matter of independent claims 16 and 22 for the reasons set out above and because Grist et al. and Aldhous fail to make up for the deficiencies in Nelson. For these reasons, the rejection under 35 U.S.C. §103 over Nelson et al., in view of Grist et al. and Aldhous should be withdrawn.

VI. Conclusion

For all of the foregoing reasons, the rejections should now be withdrawn and an early notice of all pending claims is respectfully solicited. Should the Examiner wish to discuss

any issues of form or substance in order to expedite allowance of the pending application, he is invited to contact the undersigned attorney at the number indicated below.

Respectfully submitted,

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APPENDIX A

1. A method of making a mixture of VNTR alleles and their flanking regions of the genomic DNA of one or more members of a species of interest, which method comprises the steps of:

a) dividing genomic DNA of the species of interest into fragments,

b) ligating to each end of each fragment an adapter thereby forming a mixture of adapter-terminated fragments in which each 3'-end is blocked to prevent enzymatic chain extension,

c) contacting a portion of the mixture of adaptor-terminated fragments with an adapter primer and a VNTR primer wherein said portion of the mixture of adaptor terminated fragments serves as a template to create a mixture of 5'-flanking VNTR amplimers;

d) contacting a portion of the mixture of adaptor-terminated fragments with an adaptor primer and a VNTR antisense primer wherein said portion of the mixture of adaptor terminated fragments serves as template to create a mixture of 3'-flanking VNTR amplimers,

e) and producing a desired mixture of VNTR alleles and their flanking regions by contacting genomic DNA of the one or more members of the species of interest with the mixture of 5'-flanking VNTR amplimers and/or the mixture of 3'-flanking VNTR amplimers as primers wherein said genomic DNA of the one or more members of the species of interest is used as template.

2. The method of claim 1, wherein step b) is performed by terminating each 3'-end of each fragment to prevent enzymatic chain extension, and ligating each 5'-end of each fragment to an adaptor, thereby forming a mixture of adaptor terminated fragments.

3. The method of claim 1, wherein in step c) the VNTR repeat sequences are removed from the 5'-flanking VNTR amplimers, and in step d) the VNTR repeat sequences are removed from the 3'-flanking VNTR amplimers.

4. The method of claim 1, wherein in step c) and/or d) the adaptor or primer used contains at least one phosphorothioate bond.

5. The method of claim 1, wherein step e) is performed using as primers, either successively or together, both the mixture of 5'-flanking VNTR amplimers and the mixture of 3'-flanking VNTR amplimers.

6. The method of claim 1, wherein there is used in step e) genomic DNA of one or more members of the species of interest which manifest a trait of interest, whereby the resulting mixture of VNTR alleles and their flanking sequences is representative of those which manifest the trait of interest.

7. The method of claim 6 wherein in a step f) the strands of the mixture of VNTR alleles and their flanking regions are separated and then re-annealed and any mis-matches are separated and discarded.

8. The method of claim 7, wherein step f) is repeated to recover a single VNTR allele and its flanking regions.

9. The method of claim 6, wherein at least one VNTR allele and its flanking sequences representative of those which manifest the trait of interest, is hybridised with a mixture of VNTR alleles and their flanking sequences representative of those which do not manifest the trait of interest, and at least one match and/or at least one mis-match is selected to provide at least one VNTR allele or fragment thereof which is characteristic of the trait of interest.

10. The method of claim 9, wherein the at least one VNTR allele and its flanking sequences representative of those which manifest the trait of interest, is provided with 3'-overlapping ends.

11. An isolated portion of genomic DNA of one or more members of a species of interest, said portion consisting essentially of a representative mixture of alleles of a chosen VNTR sequence and their flanking regions on both sides and which is representative of that member or members.

12. The isolated portion as claimed in claim 11, wherein the mixture of alleles is representative of those which manifest a trait of interest.

13. The isolated portion as claimed in claim 11, wherein each member of the representative mixture of alleles has an adaptor at each of its 3'-end and its 5'-end.

14. An isolated portion of genomic DNA of one or more members of a species of interest, said portion consisting essentially of a single VNTR allele and its flanking regions and an adaptor at each of its 3'-end and its 5'-end, said allele being characteristic of those which manifest a trait of interest.

15. An isolated portion of genomic DNA of a species of interest, said portion consisting essentially of a representative mixture of 3'-flanking regions of a chosen VNTR sequence, each member of the mixture carrying an adaptor at its 3'-end, and a representative mixture of 5'-flanking regions of a chosen VNTR sequence, each member of the mixture carrying the same adaptor at its 5'-end.

16. A method of treating nucleic acids which consist essentially of a mixture of polymorphic alleles, the mixture being representative of those which manifest a trait of interest, which method comprises separating and then re-annealing strands of the mixture, and separating and discarding any mis-matches.

17. The method of claim 16, wherein the mixture of polymorphic alleles is a mixture of alleles of a chosen VNTR sequence and their flanking regions.

18. The method of claim 17, wherein the method is repeated to recover a single VNTR allele and its flanking regions.

19. The method of claim 17, wherein at least one VNTR allele and its flanking sequence representative of those which manifest the trait of interest, is hybridised with a mixture of VNTR alleles and their flanking sequences representative of those which do not manifest the

trait of interest, and at least one match and/or at least one mis-match is selected to provide at least one VNTR allele or fragment thereof which is characteristic of the trait of interest.

20. The method of claim 19, wherein the at least one VNTR allele and its flanking sequence representative of those which manifest the trait of interest, is provided with 3'-overlapping ends.

21. A method of making a mixture of amplimers which method comprises the steps of:

a) dividing genomic DNA of one or more members of a species of interest into fragments,

b) ligating to each end of each fragment an adaptor thereby forming a mixture of adaptor-terminated fragments in which each 3'-end is blocked to prevent enzymatic chain extension, and

c) contacting a portion of the mixture of adaptor-terminated fragments with an adaptor primer and a VNTR primer wherein said portion of the mixture of adaptor terminated fragments serves as a template to create a mixture of 5'-flanking VNTR amplimers, and/or

d) a portion of the mixture of adaptor-terminated fragments with an adaptor primer and a VNTR antisense primer wherein said portion of the mixture of adaptor terminated fragments serves as a template to create a mixture of 3'-flanking VNTR amplimers

21. A method of making a mixture of amplimers which method comprises the steps of:

a) dividing genomic DNA of one or more members of a species of interest into fragments,

b) ligating to each end of each fragment an adaptor thereby forming a mixture of adaptor-terminated fragments in which each 3'-end is blocked to prevent enzymatic chain extension, and

c) using a portion of the mixture of adaptor-terminated fragments as templates with an adaptor primer and a VNTR primer to create a mixture of 5'-flanking VNTR amplimers, and/or

d) using a portion of the mixture of adaptor-terminated fragments as templates with an adaptor primer and a VNTR antisense primer to create a mixture of 3'-flanking VNTR amplimers.

22. A method of identifying an allele which is linked to a trait of interest, which method comprises incubating together under hybridisation conditions: at least one molecule of nucleic acid containing a polymorphic allele and its flanking sequences representative of those which manifest the trait of interest; and a mixture of molecules of nucleic acid which contain polymorphic alleles and their flanking sequences representative of those which do not manifest the trait of interest; and selecting at least one match and/or at least one mis-match to provide at least one allele or fragment thereof which is linked to the trait of interest.

23. The method of claim 22, wherein the alleles are VNTR alleles.

24. The method of claim 22, wherein the at least one allele and its flanking sequences representative of those which manifest the trait of interest, is provided with 3'-overlapping ends.

25. A method for diagnosing a trait of interest comprising the step of identifying an allele which is linked to a trait of interest according to the method of claim 22, wherein said molecules of nucleic acid are contacted with a portion of genomic DNA as claimed in claim 14.

26. The method of claim 1 or claim 16, wherein the VNTR allele and its flanking regions, or the mixture of VNTR alleles and their flanking regions, is analysed by being applied under hybridsation conditions to an array of immobilised VNTR alleles and/or their flanking regions.

27. A kit comprising protocols and reagents for performing the method of claim 1 or claim 16 or claim 24.

APPENDIX B